

Catalog Number AQ 045 AH

Part # 11968

Contents of Kit:

- 50 QuickStix Strips packed in a moisture-resistant canister
- 20X EB2 Extraction Buffer Concentrate*
- reaction vials
- transfer pipettes

*1X Extraction Buffer must be prepared from 20X Concentrate

Items Not Provided:

- Oster blender or equivalent for grinding hay
- Portable scale to weigh ground hay (ProScale CP-120 or equivalent)
- 40-mesh sieve (optional)
- Graduated cylinder†
- Sample extraction vessels (eg, disposable sample cups with lids)†

† Available through EnviroLogix as Accessories, see page 3



Use Oster Blender or equivalent

40 mesh sieve size equates to particles that are 420 microns (0.42mm) in size

Intended Use

This EnviroLogix QuickScan Kit for CP4 EPSPS Ground Alfalfa Hay detects and quantifies CP4 EPSPS protein in ground alfalfa hay at 0.1%, dependent upon the expression level of the Roundup Ready plant.

This test is intended to give the producer, purchaser, or exporter a screening method for identification of the presence of Roundup Ready alfalfa hay from ground hay samples at levels of 0.1%, based on weight/weight (w/w) ratios. The results of each sample tested are only as representative as the sample is of the entire lot, so adherence to proper principles of hay sampling is key; see "Important-Representative Sample" below.

How the Test Works

In order to test for the CP4 EPSPS protein expressed by Roundup Ready alfalfa hay, an extract of the composite hay sample must be prepared. Extracts of cored hay and ground hay are prepared for testing using different methods (see Sample Preparation section).

Each QuickStix Strip has an absorbent pad at each end. The protective tape with arrows indicates the end of the strip to insert into the sample extract. The sample travels up the membrane strip and is absorbed into the larger pad at the top of the strip. The portion of the strip between the protective tape and the absorbent pad at the top of the strip is used to view the reactions as described under "Interpreting the Results." Results are scanned and interpreted quantitatively with the EnviroLogix QuickScan System. Please avoid bending the strips.

Sample Preparation

Prepare 1X Extraction Buffer: Mix 50 mL of 20X Concentrate with 950 mL of distilled or deionized water. Store refrigerated when not in use; allow to come to room temperature before using.

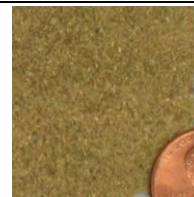
Important-Representative Sample: It is very important that the sample tested is **representative of the entire lot of hay**. A composite sample for each hay lot is required to run the test. A helpful reference is "Recommended Principles for Proper Hay Sampling" by Dan Putnam, University of California, Davis. Once a representative sample has been obtained, grind hay using an Oster blender or equivalent to a consistency that will allow ground materials to pass through a 40-mesh sieve, see illustration below showing grind quality.



Unground sample



Ground for 1 minute, contains plenty of 40-mesh particles—test the finest particles possible



Example of a sample sifted through 40-mesh sieve



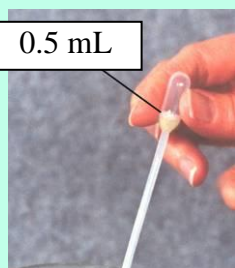
Carefully weigh out sample and measure 1X Extraction Buffer



Shake well – the sample may tend to clump together and/or adhere to the cup, so check carefully and make sure all dry material is wet



Tip sample cup if needed to avoid particulates when pipetting



0.5 mL

Transfer 0.5 mL to reaction vial

Important-Clean Between Samples: All grinding and weighing equipment, as well as testing surfaces, must be **cleaned thoroughly if a sample tests positive** to ensure there is no cross-contamination to the next sample. It is advisable that disposable pipettes and vials be stored closed and only removed when used to prevent the potential for dust affecting the performance of the test on subsequent samples. If possible, use separate rooms for grinding/weighing and testing.

1. After grinding composite sample, **remove 1 gram** and place in the extraction vessel. Use a balance to weigh out ground material carefully and avoid cross-contaminating samples with hay fines. Measure carefully and **add 80mL** of prepared 1X Extraction Buffer to the disposable sample cup containing hay and securely close the lid.
2. Shake cup for 1 minute by hand. Look at the bottom of the cup and confirm that all material is wet from the shaking process before proceeding. Continue shaking until all material is wet.
3. Draw up enough liquid portion from the settled sample to fill the long narrow tip of the transfer pipette up to the line at the top of the flared portion of the pipette bulb (see illustration, left). Avoid pulling up particles. Dispense extract (~0.5 mL) into reaction vial.

How to Run the QuickStix Strip Test

1. Allow refrigerated canisters to come to room temperature before opening. Remove the QuickStix Strips to be used. Avoid bending the strips. Reseal the canister immediately.
2. Place one strip, colored arrows pointing down, into the reaction vial containing extract. The sample will travel up the strip.
3. Allow the strip to develop for 5 minutes before making final assay interpretations.
4. Immediately cut off and discard the bottom section of the strip covered by the arrow tape and place in the QuickScan Reader. Strips must be read while still wet. Alternatively, strips may be read visually at this point. Do not save strips.

NOTE: Use extreme caution to prevent sample-to-sample cross-contamination with hay, dust, fluids, or disposables.

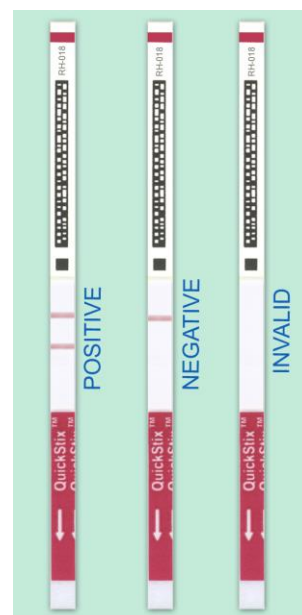
Interpreting the Results

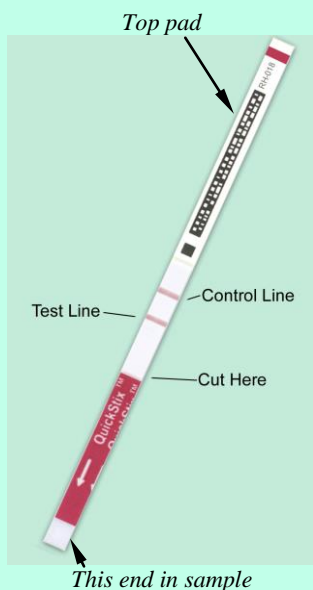
Development of the Control Line within 5 minutes indicates that the strip has functioned properly. Any strip that does not develop a Control Line should be discarded, and the sample re-tested using another strip.

Results are scanned and interpreted quantitatively with the QuickScan System. Place QuickStix into the carrier, slide in, and press “Read Test” on the screen. QuickScan will return a result as “% GMO” or “<LOD” (less than the Limit of Detection). Please consult the QuickScan User Manual for details.

The test may be used qualitatively without the use of QuickScan. A visible pink test line will appear if the sample contains 0.1% CP4 EPSPS or more.

If the extract is from a sample with less than 0.1% CP4 EPSPS, the strip will only develop the Control Line.





Kit Storage

This kit can be stored at room temperature, or refrigerated for a longer shelf life. Please note the shelf life on the kit label for each storage temperature. The kit may be used in field applications; however, prolonged exposure to high temperatures may adversely affect the test results. Do not open the desiccated canister until ready to use the test strips.

Precautions and Notes

- This kit is designed to give quantitative results using the QuickScan System and may also be visually interpreted.
- The strips will detect Roundup Ready protein in sample extracts prepared following the specified extraction procedure at composite hay levels of approximately 0.1% (w/w) Roundup Ready alfalfa or more, and is dependent upon the expression level of the Roundup Ready alfalfa plant.
- An example of a 0.1% (w/w) sample would be a mixed composite sample containing 0.1 grams of Roundup Ready alfalfa hay and 99.9 grams of conventional alfalfa hay.
- This product is currently not applicable for use in any other crop.
- As with all tests, it is recommended that results be confirmed by an alternate method when necessary.
- The assay has been optimized with the protocol and with the buffer provided in the kit. Deviation from this protocol may invalidate the results of the test.
- The results generated through the proper use of this diagnostic tool reflect the condition of the working sample directly tested. Extrapolation as to the condition of the originating lot, from which the working sample was derived, should be based on sound sampling procedures and statistical calculations which address random sampling effects, non-random seed lot sampling effects and assay system uncertainty. A negative result obtained when properly testing the working sample does not necessarily mean the originating lot is entirely negative for the analyte or protein in question.
- A negative result with this kit does not mean that the sampled tissue has not been otherwise genetically modified.
- It is not safe to conclude that a sample is negative before a full 5 minutes have elapsed.
- Protect all components from hot or cold extremes of temperature when not in use. Do not leave in direct sunlight or in vehicle.

Accessories available through EnviroLogix

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|------------------------------------|------------|-------|
| ▪ Sample cups with lids (50) | ACC 012 | 11224 |
| ▪ Sample cups with lids (500/case) | ACC 012-CS | 10167 |
| ▪ Graduated cylinder (100 mL) | ACC 068 | 11207 |





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